P124

Metabolites in Culture Media Predict Cartilage Extracellular Matrix Accumulation For Chondrocytes in Crosslinked Polypeptide Gels

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Purpose: Cell viability and matrix accumulation in scaffolds are important measurement outcomes of engineering cartilage tissues in vitro. Early assessments that predict longer-term outcomes would aid in more rapid screening of multiple scaffold-cell combinations. The objective of this study was to measure media metabolites for multiple chondrocyte-laden scaffolds at very early time points in culture, to evaluate their ability to predict extracellular matrix accumulation in the same samples at longer times.
Methods and Materials: Porcine chondrocytes were encapsulated in 16 distinct formulations of a crosslinked elastin-like polypeptide (ELP) . Constructs were cultured for 28 days, and culture media sampled on days 4 and 7 for quantification of glucose, lactate, and pyruvate. On day 28, samples were assayed for accumulated sGAG. Regressions for accumulated sGAG on each metabolite were performed and tested for statistical significance via ANOVA.
Results: Metabolite concentrations at days 4 and 7 revealed significant correlations for sGAG accumulation with all three metabolites. High levels of lactate production and pyruvate consumption were strongly correlated with formulations that supported higher sGAG accumulation at the later timepoints. Glucose consumption was not a strong predictor of long-term outcome.
Conclusions: Results suggest that metabolite concentrations in culture media at early time points serve as indicators of longer term outcomes, such as matrix accumulation. This technique may be used in the engineering of tissues in vitro to rapidly screen multiple experimental parameters for their effect on matrix accumulation, and potentially other outcome measures without the need for extended cultures.

P125

Attachment of Meniscal fibrochondrocytes to A collagen Type I scaffold induces MMP and IL-6 expression

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Purpose: The preservation of the meniscus is the most important surgical goal. The use of scaffolds colonized with meniscus cells (fibrochondrocytes) to reconstruct the defect seems to be a promising way for the treatment of a meniscus trauma.
Methods and Materials: Human meniscus cells were seeded onto a bovine collagen I matrix After 14 and 28 days in culture, the cells were analyzed for the expression of IL-1ß, IL-6, TGF-ß, TIMP-1, TIMP-3, MMP-1, and MMP-3.
Results: After 14 and 28 days of incubation on the scaffolds, the cells show the same mRNA expression levels of IL-1ß, TIMP-1, TIMP-3, and TGF-ß when compared to controls. In contrast, the IL-6 (12.7-fold ± 4.4, $p<0.001$), MMP-1 (11.3-fold ± 2.4, $p<0.001$), and MMP-3 (13.7-fold ± 6.8, $p<0.031$) were upregulated on transcription levels in the scaffold when compared to controls after the same period of culture. After 28 days of culture in scaffold the expression of MMP-3 was upregulated 78.2-fold (± 7.4, $p<0.0001$), MMP-1 (71.3-fold ± 5.9, $p<0.0001$) and IL-6 was elevated 98.9-fold (± 9.1, $p<0.0001$) compared to controls.
Conclusions: We could show that meniscus cells revealed a significantly increased expression of MMP1 and MMP-3, and also a significant elevation of IL-6 mRNA after 14 and 28 days of culture. This may lead to a destruction of the scaffold-matrix itself and the extracellular matrix of the meniscus. Secondly, IL-6 could induce a global inflammation around the scaffold. Alternatively, MMP activation may be necessary for remodelling processes during replacement of the scaffold matrix with autologous extracellular matrix.

P126

Effect of different materials on the proliferation and migration of articular chondrocytes.

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Purpose: Materials with good biomimetic properties may facilitate the initial phases of regeneration. Migration is a complex process that is important during development and tissue repair. The aim of this study was to evaluate the biomimetic properties of different materials.
Methods and Materials: Cartilage from young pigs was obtained after euthanasia. The cartilage was minced using a scalpel. Similar amount of cartilage was combined with Hyaff 11 scaffolds (Hyaff 1112, 37mg±2,41)without coating or gel coated with Puramatrix ™ or Cartipatch ™. The constructs were cultured with proliferative media for 21 days. After this period the media was changed to a chondrocyte differentiation media. The number of migrated chondrocytes was determined by measuring the amount of DNA in the different groups. The chonrocyte differentiation was assessed with the quantification of glycosaminoglycans (GAG) and the presence of collagen type II.
Results: Cells migrated and populated the constructs in all groups. Puramatrix had a significantly higher number of cells after 33 days ($p=0,0143$). All the groups evidenced collagen production. Collagen type II was only present in the puramatrix group. The amount of GAG was significantly higher in the puramatrix group. ($p=0,00270$).
Conclusions: The different three dimensional and chemical properties of materials affect important processes that are involved in tissue repair. Puramatrix evidenced to elicit better migration and differentiation capacity in pig chondrocytes.

P127

Effects of a Regionally-Specific Collagen-Based Implant on Repair of Osteochondral Defects in a Caprine Model

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Purpose: The objective was to investigate the effects of a novel implant with regionally-specific composition and structure on the repair of osteochondral defects in the goat. The distribution of proteoglycan 4 (PRG4; lubrican/superficial zone protein) in reparative tissue was included in the evaluation.
Methods and Materials: Two osteochondral defects (4mm diameter, 6mm depth) were created in the trochlear groove of one stifle joint in six adult Spanish goats. Defects in three animals (n=6 defects) were treated with a construct comprising two integrated porous layers consisting of 1) an unmineralised material based on type II collagen (thickness ~1.5mm) and 2) a mineralised material containing type I collagen, calcium phosphate and glycosaminoglycan (thickness ~ 4.5mm). Untreated control defects (n=6 defects) were left empty. At 16 weeks, implant sites were analysed histomorphometrically for the type and amount of reparative tissue and stained immunohistochemically for PRG4.
Results: Mean histomorphometric values of reparative tissues filling the chondral region of the defects suggested benefit of the implanted scaffold over untreated controls in several outcome variables (eg total reparative fill, amount of hyaline cartilage and fibrocartilage), but large variablility precluded statistical significance. Qualitative differences evident in the subchondral region of the defects indicated benefits of the implants in promoting new bone formation. PRG4 was observed in the reparative superficial zone of both implanted and untreated defects.
Conclusions: The strategy of treating ostechondral defects with a scaffold with regionally-specific composition and structure shows promise. The presence of PRG4 in the reparative tissue may benefit its tribological performance.